Development of Murine Allergic Asthma Is Dependent Upon B7-2 Costimulation[1]

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Allergic asthma is thought to be mediated by CD4+ T lymphocytes producing the Th2-associated cytokines, IL-4, and IL-5. Recently, the costimulatory molecules B7-1 and B7-2, which are expressed on the surface of APC, have been suggested to influence the development of Th1 vs Th2 immune responses. We examined the in vivo role of these costimulatory molecules in the pathogenesis of Th2-mediated allergen-induced airway hyperresponsiveness in a murine model of asthma. In this model, OVA-sensitized A/J mice develop significant increases in airway responsiveness, pulmonary eosinophilia, and pulmonary Th2 cytokine expression following aspiration challenge with OVA as compared with PBS-control animals. Strikingly, administration of anti-B7-2 mAb to OVA-treated mice abolished allergen-induced airway hyperresponsiveness, pulmonary eosinophilia, and elevations in serum IgG1 and IgE levels. Anti-B7-2 treatment of OVA-treated mice reduced both total lung IL-4 and IL-5 mRNA and bronchoalveolar lavage fluid IL-4 and IL-5 protein levels, with no significant changes in IFN-γ message or protein levels. In contrast, treatment with anti-B7-1 mAbs had no effect on allergen-induced airway hyperresponsiveness, IgE production, or cytokine production, however, it significantly suppressed pulmonary eosinophilia. We conclude that B7-2 provides the necessary costimulatory signal required for the development of in vivo allergic responses to inhaled allergen exposure. ***The Journal of Immunology,*** **1998, 160: 1036–1043.**

Allergic asthma is a disease characterized by airway hyperresponsiveness, pulmonary inflammation, and elevated serum IgE levels. Increasing evidence suggests that T lymphocytes, in particular CD4+ T cells of the Th2 phenotype, play a pivotal role in the development of the airway hyperresponsiveness and the eosinophilic inflammatory response common in asthma (1–3). Elevated IL-4 and IL-5 levels in bronchial biopsies (3), bronchoalveolar lavage (BAL)[3] cells (1, 2), and blood (2) of allergic asthmatic patients have been noted. Since these cytokines promote eosinophil chemotaxis (4), activation (5), and survival (6), as well as IgE production by B cells (7), this cytokine pattern has been thought to be important in human allergic asthma.

We have recently provided additional support for this hypothesis in a murine model of Ag-induced airway hyperresponsiveness and pulmonary eosinophilia in which allergic responses are CD4+ T cell dependent (8) and associated with increases in Th2 cytokines in the lung (9). In addition, we demonstrated that administration of rIL-12, a cytokine important in Th1 cell differentiation, both prevented and reversed the development of allergic airway responses in mice (9). Despite considerable evidence suggesting that T lymphocytes play a pivotal role in the pathogenesis of asthma, the molecular signals that direct the differentiation of naive T cells into pathogenic Th2 cytokine-producing cells in the lung in response to inhaled allergens are not well understood.

CD4+ T cell activation requires two distinct signals from APC (10). The first signal, which confers specificity, is provided by the interaction of the TCR with MHC II complexes on APC. A second costimulatory signal can be provided by APC-borne ligands for the CD28 and CTLA-4 receptors on T cells. TCR ligation in the absence of costimulation induces Ag-specific T cell anergy (11). The ligands for CD28 and CTLA-4 are B7-1 (CD80) and B7-2 (CD86) (12, 13). Blockade of the B7/CD28 pathway with CTLA4Ig, a soluble fusion protein (14), has been shown to effectively inhibit T cell activation in vitro (15, 16) and in vivo (17–22). Recently, some studies have suggested that B7/CD28-CTLA-4 interactions may not only be important in T cell activation and IL-2 production, but may also play a role in T cell differentiation with B7-1 favoring development of Th1 cells and B7-2 favoring Th2 cells (22–24). In contrast, other studies have suggested that B7-1 and B7-2 molecules can substitute for each other during Th2 differentiation (25, 26). Few studies have examined the potential role of these costimulatory molecules in the development of T cell-dependent allergic airway responses.

In the present study, we examined the relative contribution of B7-1 and B7-2 to the development of Th2-mediated allergic airway responses in an in vivo murine model of asthma, which we have previously described (8, 9). Our results demonstrate that B7-2 costimulatory molecules are required for the development of a type 2 cytokine pattern as well as the development of allergic airway responses. In contrast, treatment with anti-B7-1 did not block the course of the type 2 immune response.

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[3] Abbreviations used in this paper: BAL, bronchoalveolar lavage; HPRT, hypoxanthine guanine phosphoribosyltransferase.

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Materials and Methods

Animals

Six-week-old male A/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were housed in a laminar flow hood in a virus-free animal facility for the duration of the experiments. The studies reported here conformed to the principles for laboratory animal research outlined by the Animal Welfare Act and the Department of Health, Education and Welfare (National Institutes of Health) guidelines for the experimental use of animals (n = 6–8 mice/experimental group).

Special reagents

Abs used for the in vivo cytokine intervention experiments included rat anti-mouse B7-2 (GL1) (27), hamster anti-mouse B7-1 (16-10A1) (28), control isotype-matched rat IgG2a (GL117), and control normal hamster IgG, which were prepared as previously described (29).

Effects of B7 blockade on allergic airway responses to Ag challenge

Mice were sensitized by i.p. injection of OVA (10 μg in 0.3 ml PBS) or PBS alone (controls). Two weeks later, mice were anesthetized with a mixture of ketamine and xylazine (45 and 8 mg/kg, respectively) and challenged by aspiration. Specifically, mice are placed on a board in a supine position. The animal's tongue extended with lined forceps and 50 μl of a 1.5% solution of OVA or an equivalent volume of PBS (control) is placed on the back of the tongue. We have examined the deposition pattern of the Ag delivered in this manner by using Evans blue dye. We find that a small amount of the Ag is deposited in the trachea and the remainder is deposited in the airways. No Ag is detected in the esophagus or stomach.

OVA-treated (both sensitized and challenged with OVA) and PBS-treated mice (both sensitized and challenged with PBS vehicle) were injected i.v. with anti-B7-1 (100 μg), anti-B7-2 (100 μg), or a combination of both anti-B7-1 and anti-B7-2 mAbs (total of 200 μg) 24 h prior to OVA or PBS challenge. Control animals were administered either 100 μg hamster IgG, 100 μg IgG2a (GL117), or 100 μg of both hamster IgG and IgG2a (GL117). On the day of the challenge, Abs (100 μg/mouse) were administered with the PBS or OVA by aspiration in a total volume of 50 μl. Ninety-six hours after challenge, airway responsiveness to i.v. acetylcholine challenge was determined, the number of inflammatory cells in BAL fluids was determined, lungs were saved for measurement of cytokine mRNA levels, and blood was taken for analysis of Ig levels.

Airway responsiveness measurements

Airway responsiveness to i.v. acetylcholine challenge was measured as previously described with minor modifications (8, 9). Briefly, mice were anesthetized with sodium pentobarbital (17.5 mg/ml), intubated with a 20-gauge tracheal cannula, and ventilated at a rate of 120 breaths/min with a constant tidal volume of air (0.2 ml). Airway pressure was measured with a pressure transducer via a port of the tracheal cannula. Muscle paralysis was provided by i.v. administration of decamethonium bromide (25 mg/kg). After establishment of a stable airway-pressure recording, acetylcholine was injected i.v. (50 μg/kg) and the changes in airway pressure were recorded. Airway responsiveness was defined by the time-integrated change in peak airway pressure (airway pressure-time index; cm H_2O per s).

Assessment of airway inflammation

After airway responsiveness measurements, lungs were lavaged thoroughly with 1 ml of HBSS solution without calcium or magnesium plus 10% FBS. The lavage fluid was centrifuged (300 × g × 10 min), the supernatant was removed for cytokine analysis, and the cell pellet was resuspended in 1 ml of HBSS solution plus 10% FBS, and counted with a hemocytometer. Slide preparations were stained with Diff-Quick (Baxter, McGaw Park, IL) and BAL cell differential percentages were determined based on light microscope evaluation of >500 cells/slide.

Quantitation of cytokine mRNA levels in the lung

RNase-free plastic and water were used throughout the assay. Tissues were homogenized in RNAzol B (Cinna/Biotecs, Friendswood, TX) at 50 mg of tissue/ml. Purified RNA (10 μg) was subjected to electrophoresis on a 2% formaldehyde gel containing ethidium bromide (30). The gel was photographed and individual lanes were examined for the presence of 18S and 28S ribosomal bands, the absence of RNA degradation, and the quantity of RNA loaded onto each lane.

The procedures for reverse transcription and PCR were previously reported (30) and are briefly described here. RNA samples were reverse transcribed with Superscript RT (Bethesda Research Laboratories, Rockville, MD), and cytokine-specific primers were used to amplify selected cytokines (30). For each cytokine, the optimum number of cycles (i.e., the number of cycles that would produce a detectable quantity of cytokine product DNA that was directly proportional to the quantity of input mRNA) was determined experimentally. To verify that equal amounts of undegraded RNA were added in each RT-PCR reaction within an experiment, the "housekeeping gene," hypoxanthine guanine phosphoribosyltransferase (HPRT), was used as an endogenous internal standard, and amplified with specific primers at the number of cycles at which a linear relationship between input RNA and final HPRT product was detected. Although HPRT values did not usually vary more than two- to threefold, values for specific cytokines were normalized to HPRT values. Amplified PCR product was detected by Southern blot analysis and the resultant signal was quantitated with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Mean values are expressed relative to the means of the PBS-challenged animals, which were arbitrarily given a value of 1.

Quantitation of cytokine protein levels in BAL fluid

After airway measurements, mice were lavaged thoroughly with 1 ml of HBSS solution without calcium and magnesium plus 10% FBS. The lavage fluid was centrifuged, and aliquots of the supernatants were stored without further treatment at −80°C until analyzed by ELISA. ELISAs for IL-4, IL-5, and IFN-γ were conducted using matching Ab pairs obtained from PharMingen (San Diego, CA), according to the manufacturer's instructions. The following Ab pairs were used for ELISA detection of IL-4, IL-5, and IFN-γ, respectively: BVD4-1D11 and BVD6-24G2; TRFK5 and JES1-39D10; R46A2 and XMG1.2. OD readings of samples were converted to picograms per milliliter using values obtained from standard curves generated with varying concentrations of recombinant IL-4, IL-5 and IFN-γ (2000 pg/ml-5 pg/ml). The limit of detection was 5 pg/ml for each assay.

OVA-specific IgG1 and IgG2a ELISA assays

Sera were obtained from blood taken during exsanguination of the animals after airway measurements. IgG subclass specific ELISAs were used to quantitate OVA-specific IgG1 and IgG2a Ab levels in serum. Briefly, 96-well Corning ELISA plates were coated with 50 μl of OVA (100 μg/ml) in HBSS overnight at room temperature. Sixteen hours later, wells were blocked with the addition of PBS-10% FBS (200 μl/well) for 2 h at room temperature. Following blocking, the plates were washed with PBS-Tween-20, sera were added (100 μl/well of a 1:100 dilution in PBS-1% FBS), and plates were incubated overnight at 4°C. Plates were then washed with PBS-Tween and incubated with biotin-conjugated anti-mouse IgG1 (1:2000; γ_1 chain specific) or anti-mouse IgG2a (1:2000; γ_{2a} chain specific; PharMingen) for 1 h at room temperature. After washing, plates were blotted dry and developed with 100 μl of an avidin peroxidase solution (ABTS, Kirkegaard and Perry, Gaithersburg, MD). Plates were read at 405 nm.

Quantitation of total serum IgE

Sera were obtained from blood taken during exsanguination of the animals after airway measurements and 100 μl (1:50 dilution in 1% FBS in PBS) was added/well. An IgE-specific ELISA was used to quantitate total IgE Ab levels in serum using matching Ab pairs (R35-72 and R35-92) obtained from PharMingen according to the manufacturer's instructions. OD 405 readings of the samples were converted to picograms per milliliter using values obtained from standard curves generated with varying concentrations of IgE and the final concentration was obtained by multiplying by the dilution factor.

Data analysis

Data are summarized as mean ± SE. The statistical analyses of the results were performed by analysis of variance using Fisher's least significant difference test for multiple comparisons. Probability values <0.05 were considered significant.

Results

B7-2 blockade ablates airway hyperresponsiveness and pulmonary inflammation

As B7-1 and B7-2 molecules are thought to be important in Th cell differentiation, we sought to determine the relative contribution of B7-2 and B7-1 to the development of Th2-mediated Ag-induced airway hyperresponsiveness and inflammation. As previously reported (8, 9), airway reactivity to i.v. acetylcholine challenge in OVA-treated mice was significantly increased

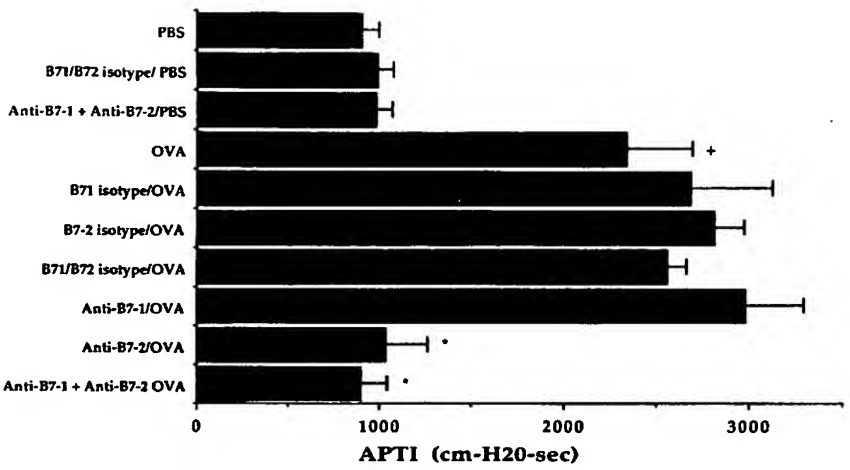


FIGURE 1. B7-2 blockade inhibits Ag-induced airway hyperresponsiveness to i.v. acetylcholine in mice 96 h after a single challenge with OVA or PBS. OVA-sensitized mice were injected i.v. with either anti-B7-1 (100 μg), anti-B7-2 (100 μg), or a combination of both Abs (200 μg) 24 h prior to aspiration challenge with OVA, respectively. Control animals were administered either 100 μg hamster IgG, 100 μg IgG2a (GL117) or 100 μg of both hamster IgG and IgG2a (GL117). On the day of the challenge, either specific Abs or isotype-matched control Abs (100 μg/mouse) were administered with the PBS or OVA by aspiration in a total volume of 50 μl. Values shown are means + SE of six to eight animals per group. $^{+}p < 0.05$ compared with PBS group; $^{*}p < 0.05$ compared with OVA + respective isotype control mAb-treated groups.

compared with that in PBS-treated mice (Fig. 1). Strikingly, anti-B7-2 treatment completely ablated airway hyperresponsiveness to acetylcholine in OVA-treated mice as compared with airway responses of OVA-treated mice receiving the isotype-matched Ab (GL117). In marked contrast, anti-B7-1 mAb treatment had no significant effect on airway reactivity in OVA-treated mice as compared with their controls (OVA + hamster IgG). Isotype-matched control Ab (i.e., hamster IgG or rat IgG2a (GL117)) administration had no significant effect on airway reactivity in OVA-treated mice. Combining anti-B7-1 and anti-B7-2 mAb treatments suppressed airway responses to the same degree as anti-B7-2 mAb treatment alone. These results demonstrate the importance of B7-2 molecules in the functional airway responses to T cell activation by inhaled Ags.

Examination of the cellular composition of BAL fluids revealed that the majority of cells in the BAL fluid of PBS control animals were alveolar macrophages (Fig. 2*A*). Following OVA sensitization and challenge no significant increases in BAL macrophages were observed, however, there were marked increases in the number of BAL eosinophils (Fig. 2*B*). Anti-B7-2 treatment of OVA-treated mice resulted in virtual ablation of eosinophils in the BAL, while anti-B7-1 partially suppressed the number of eosinophils recovered in the lavage fluid. The combination of anti-B7-1 and B7-2 mAbs did not result in any additional significant inhibitory effect on eosinophil numbers over that observed with anti-B7-2 treatment alone. All three isotype-matched control Abs had a tendency to reduce alveolar macrophage numbers although not significantly; however, they had no significant effect on eosinophil numbers in the BAL.

B7-2 costimulation is required for Th2 cell differentiation

To determine the role of B7 molecules in T cell differentiation and cytokine production, we examined the effects of blockade of B7-1, B7-2, or both on IL-4, IL-5, and IFN-γ mRNA and protein levels. Detectable mRNA and protein levels of IL-4, IL-5, and IFN-γ were observed in PBS-treated mice (Figs. 3, 4, and 5). As we have previously demonstrated (9), following Ag challenge significant increases in both mRNA and protein levels of the type 2 cytokines, IL-4 and IL-5, were observed, whereas no significant increases in the type 1 cytokine, IFN-γ were found. Ag challenge also induced significant increases in IL-10 mRNA levels as compared with PBS controls (Fig. 6). Anti-B7-2 or the combination of anti-B7-1 and anti-B7-2 mAbs significantly suppressed Ag-induced increases in protein levels of IL-4 and IL-5 as compared with their respective isotype Ab controls, while having no effect on IFN-γ levels. In marked contrast, anti-B7-1 did not significantly alter IL-4, IL-5, or IFN-γ mRNA or protein levels in OVA-treated mice as compared with hamster IgG treatment. Interestingly, the combination of anti-B7-1 and anti-B7-2 Abs significantly increased BAL IFN-γ protein levels in PBS-treated mice. Isotype control Ab administration to OVA-treated mice had no significant effect on BAL IL-4 and IL-5 protein levels as compared with OVA-treated mice receiving no Ab treatments. These results demonstrate that B7-2 provides costimulation for the differentiation of T cells into type 2-producing cells in response to inhaled Ag, and suggests that B7-1 does not contribute to the immune response in this model, at least to the extent that the dose of anti-B7-1 Ab used inhibited B7-1 interactions.

Effects of B7 blockade on serum Ig levels

To examine the role of B7 molecules in humoral responses to inhaled OVA exposure, we measured OVA-specific levels of IgG1 and IgG2a and total IgE in serum samples from animals receiving Abs to B7-1, B7-2, or both. Administration of anti-B7-2 mAb to Ag-treated mice resulted in significant suppression of OVA-specific IgG1 when compared with mice treated with the isotype control Ab GL117, while having no effect on IgG2a levels (Fig. 7). On the other hand, anti-B7-1 did not affect either IgG1 or IgG2a levels. Combined mAb treatment did not result in any significant inhibitory effect on Ig levels over that observed with anti-B7-2 treatment alone. Isotype-matched control mAb treatment had no effect on OVA-specific serum levels of IgG1 or IgG2a.

OVA sensitization and challenge resulted in significant elevations in total serum IgE levels as compared with PBS controls (Fig. 8). Anti-B7-2 mAb treatment abolished OVA-induced increases in IgE levels when compared with isotype control Ab-treated mice, while anti-B7-1 had no effect. Isotype-matched control Abs had no significant effect on IgE levels in either PBS- or OVA-treated mice. As IgE Ab production is IL-4 dependent, these results lend further support for the role of B7-2 costimulatory molecules in

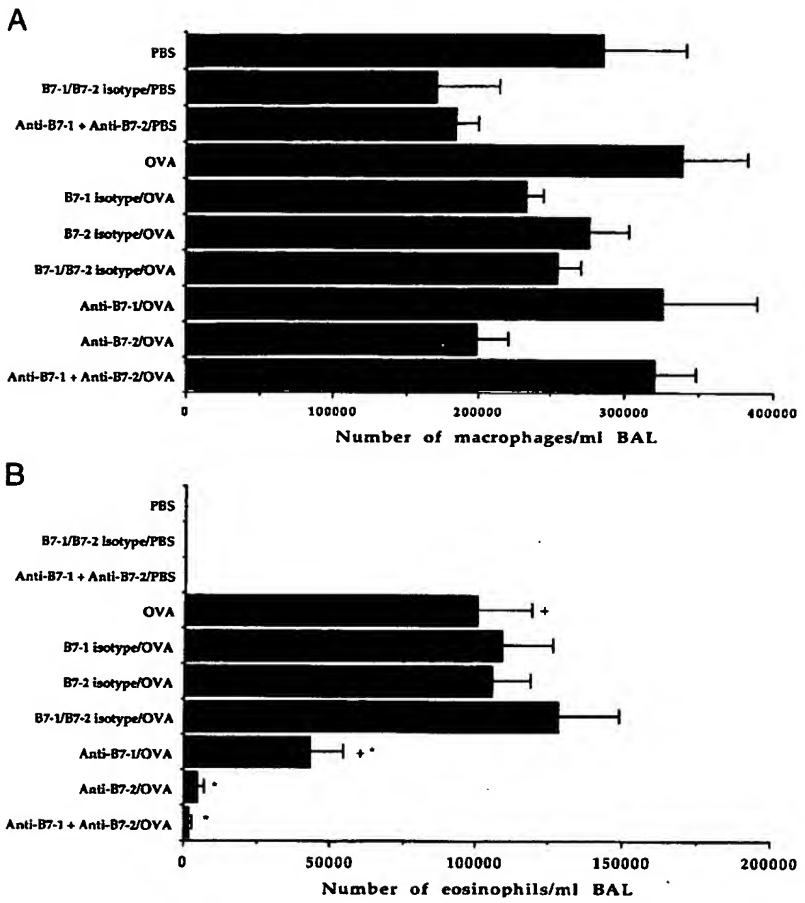


FIGURE 2. Effects of B7 blockade on numbers of BAL macrophages (*A*) and eosinophils (*B*) recovered from mice 96 h after a single OVA or PBS challenge. Values shown are mean + SE of 6 to 8 mice/group. Mice were treated as described in Figure 1. $^{+}p < 0.05$ compared with PBS group; $^{*}p < 0.05$ compared with OVA + respective isotype control mAb-treated groups.

type 2 cytokine production as well as in the development of allergic responses.

Discussion

Allergic asthma is characterized by airway hyperresponsiveness, eosinophilic inflammation and elevated serum IgE levels. Several studies have shown that this disease is associated with elevated T lymphocytes in the lungs and a Th2 pattern of cytokine production (1–3). Utilizing a murine model, we have previously demonstrated that the development of allergic responses to allergens is $CD4^{+}$ T cell dependent (8) and ameliorated by the addition of agents that either directly block Th2 cytokine activity or that prevent the expansion of type 2 cytokine-producing cells (9, 31). Studies in several experimental systems have demonstrated the importance of IL-4 and IL-5 in the development of airway hyperresponsiveness, eosinophilia, and elevations in IgE (32, 33).

It has recently been demonstrated that T cell activation requires multiple signals in addition to those conveyed through interaction of the TCR with MHCII complexes on APC (10). One of the most well characterized costimulatory signaling pathways involves the CD28 and CTLA-4 molecules that are expressed on T cells and their ligands, B7 molecules that are expressed on APC (11–13). We have recently demonstrated that CTLA4Ig, a fusion protein that inhibits the interaction of B7 molecules with both CD28 and CTLA-4, ablated the development of airway hyperresponsiveness, eosinophilic inflammation, and elevations in serum IgG1 and IgE Abs when administered either prior to sensitization or prior to local lung challenge (34). Inhibition of these responses was associated with suppression of Th2 cytokines without any changes in the Th1 cytokine, IFN-γ. These studies demonstrated that B7 molecule interaction with either CD28 or CTLA-4 was important both in the initial sensitization step and in the subsequent challenge response in the lung. Our findings support that of previous studies using similar murine models (35, 36). To date at least two members of the B7 family have been identified, namely B7-1 (CD80) and B7-2 (CD86) (12, 13). Considerable controversy exists as to whether these molecules are interchangeable or mediate distinct functions through CD28 (22–26). Some studies have supported the concept that they mediate distinct functions and moreover that B7-1 is important in Th1 cell differentiation and that B7-2 molecules are important in Th2 cell differentiation (22–24). However, previous in vitro studies have suggested that either B7-2 costimulation promotes the differentiation of naive T cells toward a Th2 phenotype (24) or that B7-1 or B7-2 interactions with CD28 can support Th2 cell differentiation (25). The present study was undertaken to determine whether the ligation of B7-1, B7-2, or both molecules was required for development of a type 2 mucosal immune response to Ag exposure in a murine model.

Our results demonstrate that B7-2, but not B7-1, costimulation is necessary for the development of allergic responses to inhaled Ags in this murine model. Strikingly, B7-2 blockade completely eliminated Ag-induced airway hyperresponsiveness, eosinophilic inflammation, and elevations in serum IgE levels. In conjunction with its inhibitory effects on these functional responses, anti-B7-2 mAb ablated Ag-induced increases in Th2 cytokines in BAL

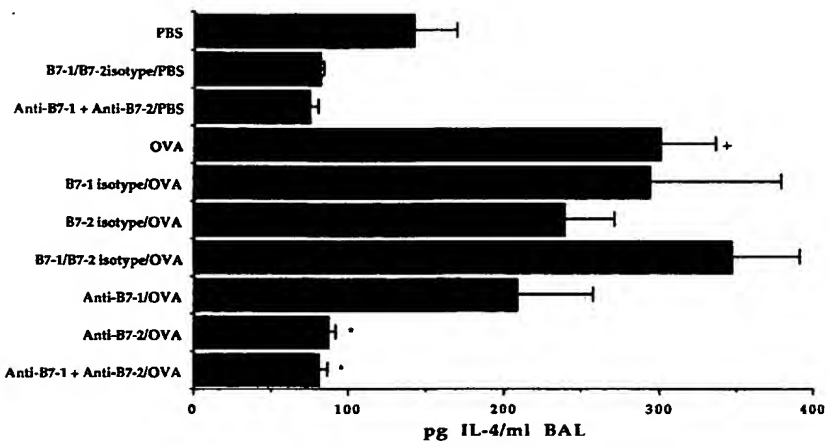


FIGURE 3. Effect of B7 blockade on IL-4 protein levels in BAL supernatants of mice 96 h after a single OVA or PBS aspiration challenge. PBS- or OVA-challenged mice were treated with anti-B7-1, anti-B7-2, or a combination of both Abs as described in Figure 1. Protein levels were analyzed using ELISAs as described in *Materials and Methods*. After subtracting background absorbance, OD readings were converted to picograms per millimeter by comparison with standard curves. Results shown are means + SE of cytokine protein levels for six to eight mice in each group. $^{+}p < 0.05$ compared with PBS group; $^{*}p < 0.05$ compared with OVA + respective isotype control mAb-treated group.

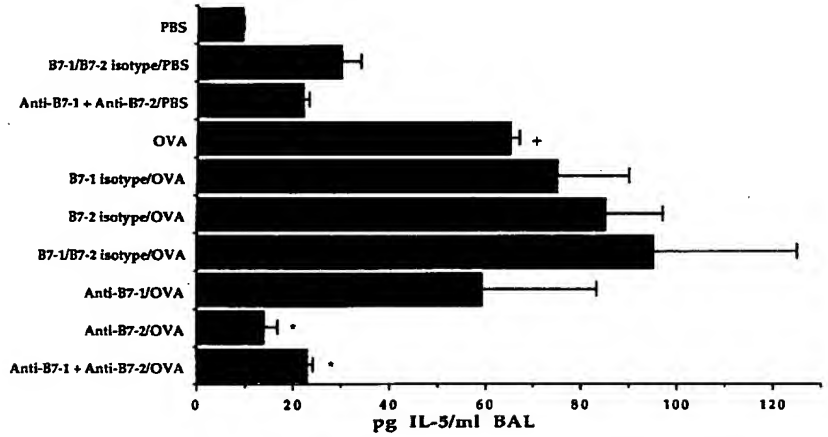
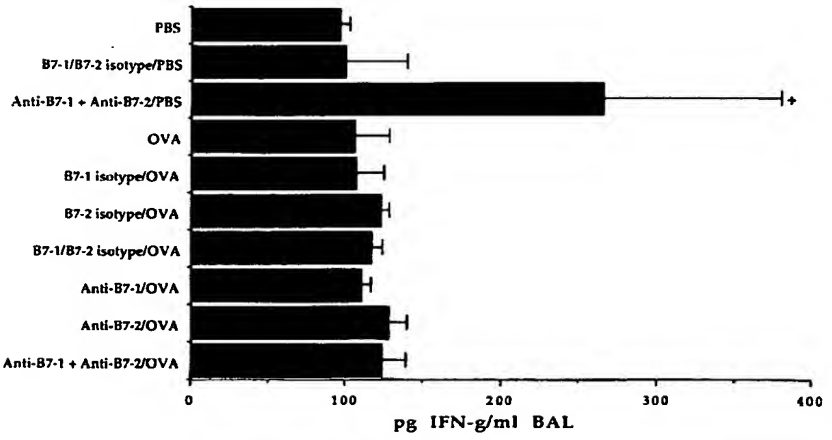


FIGURE 4. Effect of B7 blockade on IL-5 protein levels in BAL supernatants of mice 96 h after a single OVA or PBS aspiration challenge. PBS- or OVA-challenged mice were treated with anti-B7-1, anti-B7-2, or a combination of both Abs as described in Figure 1. Protein levels were analyzed using ELISAs as described in *Materials and Methods* and in the legend to Figure 3. Results shown are means + SE of cytokine protein levels for six to eight mice in each group. $^{+}p < 0.05$ compared with PBS group; $^{*}p < 0.05$ compared with OVA + respective isotype control mAb-treated group.

FIGURE 5. Effect of B7 blockade on IFN-γ protein levels in BAL supernatants of mice 96 h after a single OVA or PBS aspiration challenge. PBS- or OVA-challenged mice were treated with anti-B7-1, anti-B7-2, or a combination of both Abs as described in Figure 1. Protein levels were analyzed using ELISAs as described in *Materials and Methods* and in the legend to Figure 3. Results shown are means + SE of cytokine protein levels for six to eight mice in each group. $^{+}p < 0.05$ compared with PBS group; $^{*}p < 0.05$ compared with OVA + respective isotype control mAb-treated group.



fluids, with no effect on IFN-γ levels. As there were no significant increases in IFN-γ after Ag challenge, it is not surprising that anti-B7-2 mAb treatment did not affect IFN-γ levels. In contrast, anti-B7-1 blockade did not result in suppression of Ag-induced airway hyperresponsiveness, IgE production, or Th2 cytokine production. These results provide evidence that B7-2 costimulation can play an essential role in the development of allergic asthma.

Although B7-2 was required for the development of this type 2 allergic immune response, blocking B7-2 interactions did not cause immune deviation toward increased IFN-γ expression, nor did blocking B7-1 interactions promote a more severe allergic response. Similarly, we have shown that CTLA4Ig treatment of OVA-sensitized and challenged A/J mice suppressed Ag-induced Th2 cytokine production, but did not alter IFN-γ levels (37). Our results are in contrast to those of Tsuyuki et al.

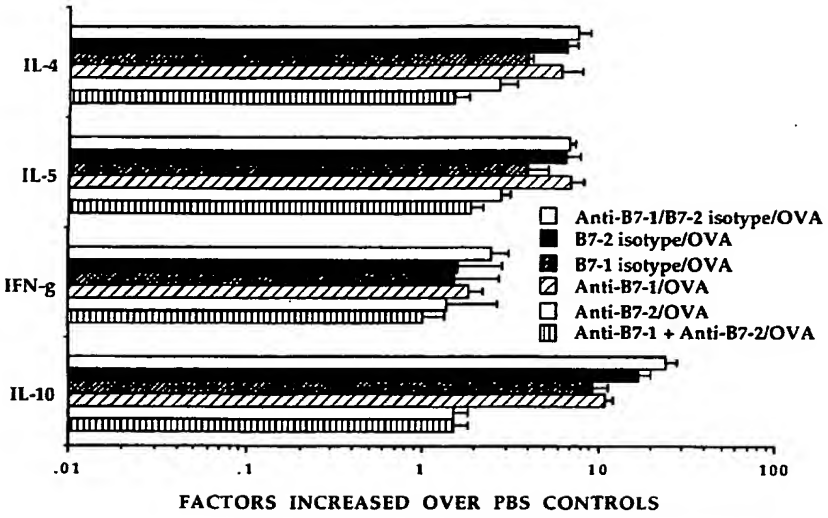


FIGURE 6. Effects of B7-1 and B7-2 blockade on IL-4, IL-5, IFN-γ, and IL-10 mRNA expression in the lungs of mice 96 h after OVA challenge. Mice were treated as described in Figure 1. RNA was extracted from lungs and purified using the RNAzol B method, reverse transcribed into cDNA, and then subjected to varying cycles of PCR as described in *Materials and Methods*. Amplification of HPRT was performed as a control for the total amount of cDNA used in PCR. Data are expressed as the mean + SE of the fold increase over control PBS values (n = 4).

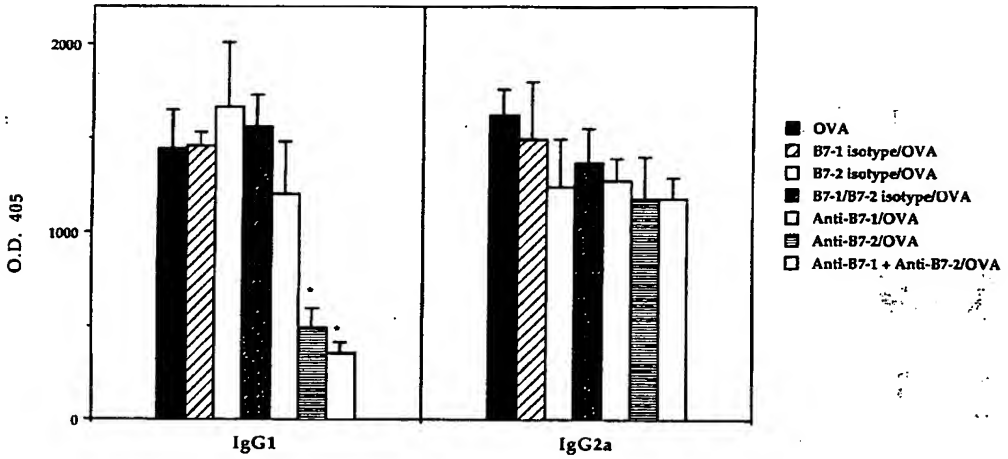


FIGURE 7. Effects of B7 molecule blockade on OVA-specific serum levels of IgG1 and IgG2a. Mice were treated as shown in Figure 1. OD values from PBS-challenged control mice were at background levels (50 ± 0.05). Values shown are the mean + SE of Ab levels from six to eight mice per group. $*p < 0.05$ as compared with respective isotype control mAb-treated OVA group.

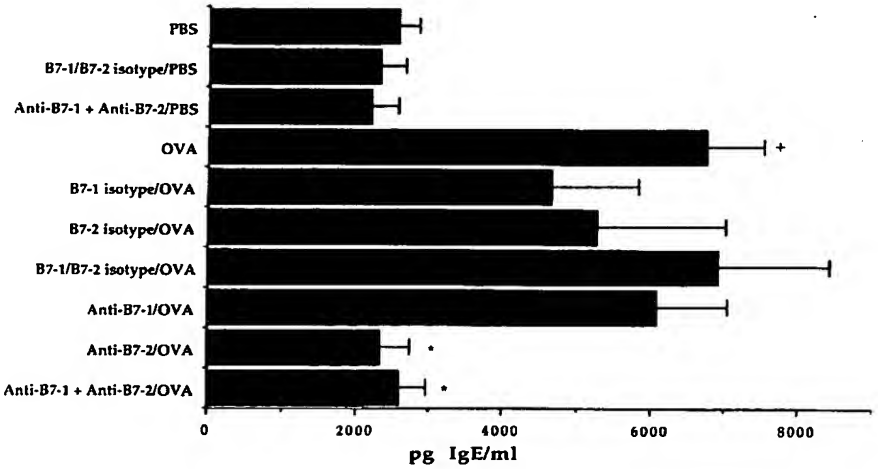


FIGURE 8. B7-2 blockade inhibits Ag-induced increases in total serum IgE levels. Mice were treated as described in Figure 1. Values are mean + SE of IgE levels from six to eight mice per group. $^{+}p < 0.05$ compared to PBS group; $*p < 0.05$ compared with respective isotype control mAb-treated OVA group.

(35) who demonstrated using a similar Ag exposure model with SV129 mice that anti-B7-2 mAb administration did in fact induce increases in IFN-γ levels in in vitro-activated lung T cells. These differences may reflect the inherent susceptibility of the A/J strain to the development of Th2 responses and that IFN-γ production or pathways associated with IFN-γ production are

deficient in these mice. Consistent with this is our finding that if these animals are given exogenous IL-12 they will produce IFN-γ, which effectively ablates the development of the allergic phenotype (9). Another potential difference is that in our study cytokines were measured in BAL fluids taken at the time of measurement of allergic airway responses whereas in their study, IFN-γ levels were derived from isolated lung cells stimulated in vitro with anti-CD3 Abs.

Our studies demonstrate that B7-2, but not B7-1, costimulation is necessary for T cell help leading to B cell activation and secretion of IgG1 and IgE. Since these Abs are associated with Th2 cell effector function, they provide further evidence that B7-2 costimulation is required for IL-4 production and the development of a type 2 immune response (7). These observations are consistent with our previous finding that CTLA4Ig prevents the development of humoral responses in allergen-challenged A/J mice (34). Consistent with the lack of increase in IFN-γ production in anti-B7-2-treated animals, IgG2a levels, which are thought to be IFN-γ dependent, were not affected by anti-B7-2 treatment. The lack of involvement of B7-1 in OVA-induced Ab production was also demonstrated using Y100F-Ig, which recognizes B7-1 not B7-2, in a murine model of Ag-induced airway eosinophilia (37).

One interesting finding was that CTLA4Ig administration to mice before Ag sensitization resulted in blockade of OVA-specific Abs of all isotypes studied (IgE, IgG1, and IgG2a), whereas when it was administered prior to Ag challenge it suppressed only IgE and IgG1 with no effect on IgG2a levels. Administration of anti-B7-2 Abs reproduced the effect of CTLA4Ig on Ab production when it was given prior to local lung challenge. These results suggest that perhaps IgE and IgG1 Ab increases were due to Ag presentation in the lung following local Ag challenge and that a more mixed response occurs during systemic sensitization. This is consistent with our observation that IgG1 and IgE Ag production is markedly elevated following local Ag challenge as compared with that following systemic sensitization alone (M. Wills-Karp and A. M. Keane-Myers, unpublished observations). This hypothesis is supported by a recent report by Chvatchko et al. (38) in which they demonstrated the formation of germinal centers within the lung parenchyma, which predominantly produced OVA-specific IgG1 and IgE in mice challenged intratracheally with OVA.

Surprisingly, anti-B7-1 treatment of OVA-sensitized and challenged animals partially suppressed OVA-induced increases in pulmonary eosinophilia, but not to the extent that anti-B7-2 treatment alone did. This finding is consistent with the reports of other investigators using similar murine models of allergic airway responses (37). Harris et al. demonstrate that blockade of B7-1 with a mutant form of CTLA4Ig, which specifically blocks B7-1, significantly reduced Ag-induced tissue eosinophilia, but had no effect on blood eosinophilia or IgE levels (37). In contrast to the partial suppression of eosinophilia by anti-B7-1 Abs, B7-2 blockade virtually ablated Ag-induced increases in BAL eosinophils. This inhibition was concomitant with the suppression of both IL-4 and IL-5 BAL levels. On the other hand, blockade of B7-1 did not inhibit Ag-induced increases in either IL-4 or IL-5 levels. This effect was also not mediated via increases in IFN-γ as no increases in IFN-γ levels were observed following anti-B7-1 treatment of OVA-sensitized animals in our study. These results suggest that the partial inhibitory effect of anti-B7-1 is due to non-IL-5 mediated processes, which contribute specifically to recruitment of eosinophils into tissues such as the production of RANTES and/or eotaxin (39, 40).

As blockade of B7-2 2 wk after the initial sensitization was effective at suppressing the development of this type 2 immune response, our results suggest that B7 costimulatory molecules are required for the activation of T cells during this secondary response. Previous in vitro (41) and in vivo (22, 42) studies have suggested that B7 costimulatory molecules may not be required for effector and memory T cell activation and cytokine production. Our results suggest that B7-2 molecules play a pivotal role in triggering of the Th2 challenge immune response in this murine model of allergy, suggesting that costimulatory signals may be important at these later stages of T cell differentiation. Consistent with this hypothesis, Finck et al. (19) has recently shown that CTLA4Ig administration can markedly ameliorate disease severity in a murine model of lupus, if administered subsequent to the development of disease. Although this effect may also be attributed to the inhibition of newly recruited T cells during this chronic disease, it is unlikely that this is the case in our allergy model, given the acute effect, within 96 h, of blocking B7-2 interactions.

Consistent with our findings, the importance of B7-2 in the differentiation of Th2 cells has also been recently demonstrated in an in vivo model of experimental allergic encephalomyelitis (23). In this model, Th1 cytokine-producing cells are associated with the disease phenotype. Anti-B7-1 treatment (resulting in Th2 cell expansion) ameliorated the disease, whereas anti-B7-2 exacerbated the disease, presumably resulting in the expansion of Th1 cells. In contrast, type 2 immune responses in response to infection with the nematode parasite, *Heligmosomoides polygyrus*, require both B7-1 and B7-2 costimulation, as blockade of both B7-1 and B7-2 was required to inhibit *H. polygyrus*-induced type 2 immune responses such as increases in serum IgG1 and IgE levels, the expansion of lymph node germinal centers, elevated blood eosinophils, and increased mucosal mast cells (43). These studies indicate that either B7-1 or B7-2 ligand interactions can provide the required costimulatory signals that lead to T cell effector function during a type 2 in vivo immune response. Thus it is clear that the requirements for costimulatory molecules during immune responses are quite complex and are likely to depend on a number of factors unique to each specific type of immune response.

The distinct requirements for B7-2 costimulation observed in this model are likely due to differential expression of B7-1 and B7-2, as other investigators have recently shown that both lung macrophages (37) and lung B cells (35) express predominantly B7-2 following inhalational exposure to OVA of mice of two different genetic backgrounds. One possible explanation for this differential expression of B7 molecules is that previous studies have indicated that there are clear differences in the kinetics of expression of B7-1 and B7-2 during an immune response (44, 45). B7-2 is constitutively expressed on monocytes and rapidly up-regulated on B cells and dendritic cells following activation. In contrast, B7-1 expression is up-regulated later during activation of the immune response on each cell type. However, in the studies described above, B7 expression was examined several weeks after the initial sensitization, which should have been sufficient time for B7-1 expression to be up-regulated. However, as we have previously shown in a similar model that systemic sensitization alone is not sufficient to elicit allergic responses in the lung, the time following local lung challenge with the immunogen may be more critical (8). Alternatively, either the nature of the immunogen, the route of exposure and/or the dose of the immunogen may influence not only the type of immune response but the degree of costimulation required (46, 47).

In summary, our studies provide compelling evidence that the development of Th2 cytokine patterns and subsequent development of allergic airway responses to inhaled Ag challenge requires B7-2 costimulation. As a predominant Th2 cytokine pattern has been observed in human asthmatics (1–3), these studies suggest

that blockade of B7-2 may provide a novel therapeutic approach to the treatment of allergic airway disorders.

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